Remarks

After entry of the foregoing amendments, claims 1, 3-10, 12-13, 15-21, 25-37 and 45-55 remain pending in the application. Claims 2, 11, 14, 22-24 and 39 have been cancelled. Claims 1, 3, 6-7, 12-13, 15-20, 25, 28, 31-32, 34-35, 38, 40, 41 and 43-45 have been amended to more clearly define the present invention. Applicants submit that no new matter has been added by these amendments. In addition, new claims 46-55 have been added. Applicants submit that these claims are fully supported by the specification.

New FIG. 21 has been added. FIG. 21 is supported on pages 152-157 of the specification in Example 3(g). No new matter has been added. Original claims 1-45 stand variously rejected under 35 U.S.C. §112, first and second paragraph, §102 and §103. Applicant respectfully requests reconsideration of the rejections and withdrawal thereof in view of the foregoing amendments and following remarks.

Priority

Applicants have inserted a paragraph containing the appropriate cross-reference as suggested by the examiner.

Drawings

The drawings stand objected to for failing to comply with 37 C.F.R. §1.84(p)(4) because reference character "figure 24" was used to designate both the figure on page 24/32 and the figure on page 26/32.

Applicants have cancelled the figure on page 24/32 and have added FIG. 21 as indicated above. Applicants respectfully submit that these amendments have obviated this rejection and request that it be withdrawn.

Specification

Applicants have added the appropriate section headings as suggested by the examiner.

Claims Rejections Under §112, First Paragraph

Claims 1-44 stand rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The examiner asserts that there is no definition in the specification for the possible heterocyclic groups in the definition of R as claimed.

Applicants respectfully submit that one skilled in the art would understand the heterocyclic groups included in the definition of R. This term is clearly defined in the specification as an aryl group containing a heteroatom, also called a heteroaryl group. Moreover, see *Dictionary of Science and Technology*, p. 523 (Professor Peter M. B. Walker ed., Larousse p/c (1995); copy of pertinent pages attached hereto) which defines "heterocyclic compounds" (*Chem.*) as "cyclic or ring compounds containing carbon atoms and other atoms, e.g., O, N, S, as part of the ring." (See also p. 63 for definition of "aryl".) Applicants respectfully request that this rejection be withdrawn as the term "heterocyclic group" is enabled by the specification.

The examiner also asserts that there is no definition in the specification for "functional group." Applicants respectfully submit that this term is also understood by those skilled in the art and therefore is adequately enabled. The *Dictionary of Science and Technology* (p. 457) defines "functional group" (*Chem.*) as "a small cluster of linked atoms with chemically active bonds." Further, the specification lists carbonyl and hydroxy groups as two examples. Other functional groups are

immediately apparent to one skilled in the art. Therefore, applicants respectfully request that this rejection be withdrawn.

Claim Rejections Under §112, Second Paragraph

Claims 1-45 stand variously rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

First, claims 1 to 44 are considered to be vague and indefinite in that it is not known what is meant by "conjugated system". Applicants submit that one skilled in the art would understand that "a conjugated system" means a chemical system in which two or more double bonds alternate with single bonds in an unsaturated system. Applicants, therefore, respectfully request that this rejection be withdrawn.

Claim 1-6, 11, 13, 14, 20, 21, 24-27 are considered to be vague and indefinite because it was not known what was meant by "-O–)$CH_2)_p$–O-" in the definition of R_7 and R_8 when taken together. Applicants submit that this rejection has been obviated by claim amendments which corrected the typographical error. Therefore, applicants respectfully request that this rejection be withdrawn.

Claim 2 was rejected for lack of antecedent basis for the phrase "COR^C, $CONH_2$, $CONHR^C$, $CONR^C{}_2$, Cyano or phosphonate". Applicants submit that this rejection has been obviated by amendment of claim 1 to incorporate the above phrase and cancellation of claim 2. Applicants, therefore, respectfully request that this rejection be withdrawn.

Claim 4 was rejected for lack of antecedent basis for the phrase "CH_2OR". Applicants respectfully submit that there is sufficient antecedent basis for the limitation "CH_2OR" in the definition of R_2 in claim 4. Claim 4 is dependent on claim 3, which is dependent on claim 1. In claim 1, R_2 is defined as "R, OH, OR, CO_2H,

CO_2R, COH, COR, SO_2R, CN." Because R is further defined as "a lower alkyl group having 1 to 10 carbon atoms . . . and optionally containing one or more hetero atoms which may form part of, or be, a functional group," there is sufficient antecedent basis for this limitation. Applicants, therefore, respectfully request that this rejection be withdrawn.

Claim 5 was rejected for lack of antecedent basis for the phrase "CH_2OAc". Applicants also respectfully submit that there is sufficient antecedent basis for the limitation "CH_2OAc" in the definition of R_2 in claim 5. Claim 5 is dependent on claim 4, which is dependent on claim 3, which is dependent on claim 1. As discussed above, there is sufficient antecedent basis for "CH_2OAc" in the definition of R in claim 1.

Claim 6 was rejected for lack of antecedent basis for the phrase "conjugated system with the double bond of the C-ring." Applicants have amended claim 6 to more clearly define its invention. As amended, applicants submit that claim 6 has antecedent basis. Applicants therefore, respectfully request that this rejection be withdrawn.

Claims 12, 19 and 28 were rejected for lack of antecedent basis for the limitation "1 to 12" in the definition of p. Applicants respectfully submit that this rejection has been obviated by the amendments to claims 12, 19 and 28 and therefore request that this rejection be withdrawn.

Claim 13 has been rejected for lack of antecedent basis for the phrase "sibirosamine pyranoside". Applicants respectfully submit that this rejection has been obviated by the amendments to claim 13, and therefore, respectfully request that this rejection be withdrawn.

Claim 16 was rejected as vague and indefinite in that the definition of R_6, R_7, R_9 and R_8 contained a typographical error. Applicants respectfully submit that this rejection has been obviated by the amendments to claim 16, and therefore, request that this rejection be withdrawn.

Claim 24 has been rejected for lack of antecedent basis for the phrase "the dimer". Applicants respectfully submit that this rejection has been obviated by the cancellation of claim 24, and therefore, request that this rejection be withdrawn.

Claim 25 has been rejected as indefinite because the claim language does not clearly set forth the metes and bounds of patent protection desired. Applicants respectfully submit that this rejection has been obviated by the amendments to claim 25, and request that this rejection be withdrawn.

Claim 30 has been rejected for lack of antecedent basis for the limitation "nitrogen protecting group" in the definition of R'_8 and R''_8. Applicants respectfully submit that the term "nitrogen protecting group" is understood by one skilled in the art. One skilled in the art would be able to ascertain which nitrogen protecting groups fall within the scope of R. The failure to provide explicit antecedent basis for the term "nitrogen protecting group" does not render claim 30 indefinite, because the scope of the claim is reasonably ascertainable by those skilled in the art. MPEP §2173.05(e) (citing *Ex Parte Porter*, 25 U.S.P.A. 2d 144, 1145 (Bd. Pat. App. & Inter. 1992)). Applicants therefore respectfully request that this rejection be withdrawn.

Claim 31 has been rejected for lack of antecedent basis for the limitation "electron withdrawing group" in the definition of R_7. Claim 31 has been amended to correct an error. As amended, claim 31 is now directed to a compound according to claim 29, wherein R_7 is an electron donating group. Applicants therefore respectfully request that this rejection be withdrawn.

Claim 36 has been rejected for lack of antecedent basis for the limitation "optionally substituted by one or more halo, hydroxy, amino, or nitro groups" in the definition of R. Applicants respectfully submit that there is sufficient antecedent basis for this limitation in claim 36. Claim 36 is dependent on claim 35, which, as amended, is dependent on claims 1, 13, 20 or 29. One of ordinary skill in the art would understand that R may be an alkyl group that is "optionally substituted by one or more halo, hydroxy, amino or nitro groups." Applicants therefore respectfully request that this rejection be withdrawn.

Claims 38 and 40-43 stand rejected as undefinite because they recite a use, without any active, positive steps defining how the use is actually practiced. Applicants respectfully submit that this rejection has been obviated by the amendment of claims 38 and 40-43 to method of treatment claims, and therefore, request that this rejection be withdrawn.

Claims 43 and 44 are rejected as vague and indefinite as a result of a typographical error in the claims. Applicants respectfully submit that this rejection has been obviated by the amendment correcting the error in claims 43 and 44. Therefore, applicants request that this rejection be withdrawn.

Claim 45 has been rejected for lack of antecedent basis for the limitation "SJG 136". Applicants submit that the amendments to claim 45 have obviated this rejection and request that it be withdrawn.

Claim Rejections Under §101

Claims 38 and 40-43 stand rejected under 35 U.S.C. §101 because the claimed use, without setting forth any steps involved in the process, results in an improper process claim. Applicants submit that this rejection has been obviated by the amendment of claims 38 and 40-43 to set forth the steps involved in the process.

Claim Rejections Under §102

Takanabe et al.

Claims 20 and 38-44 stand rejected under 35 U.S.C. §102(b) as being anticipated by Takanabe et al. (U.S. Patent No. 4,185,016). The examiner states that Takanabe et al. teaches the compounds, compositions and method of use of the compounds of Formula III where R_6 and R_7 are hydrogen, R_8 is hydrogen, methyl or methoxy and R_9 is hydroxy or acetoxy. Applicants have amended claim 20 to require that one of R_6, R_7, R_8 or R_9 is NH_2. Nowhere in Takanabe et al. is it taught or suggested that one of R_6, R_7, R_8 or R_9 should be NH_2. Therefore, applicants respectfully request that the rejection over Takanabe et al. be withdrawn.

Arima et al.

Claims 13-15, 17 and 38-44 stand rejected under §102(b) as being anticipated by Arima et al. (GB 1 299 198). The examiner cites Arima et al. as teaching the compounds, compositions and method of use of the compounds of formula II where R'_2 is CH-Me, R_6 and R_7 are hydrogen, R_8 is 4-bromobenzoyloxy and R_9 is hydrogen. Applicants have amended claim 13 so that R'_2 is O. Nowhere in Arima et al. is it taught or disclosed that R'_2 is O. Therefore, applicants respectfully request that the rejection over Arima et al. be withdrawn.

Fujisawa Pharmaceutical Company

Claims 13-15, 17 and 38-44 stand rejected under 35 U.S.C. 102(b) as being anticipated by Fujisawa Pharmaceutical Company (JP 57 131791). The examiner states that Fujisawa teaches the compounds, compositions and methods of use of the compounds of Formula II where R'_2 is O, R_6 is hydrogen, R_7 is methoxy, R_8 is hydroxy and R_9 is hydrogen. Applicants have amended claim 13 so that the compound is a dimer. Nowhere in Fujisawa is it taught or disclosed that the

compounds of Formula II may be dimers. Therefore, applicants respectfully request that the rejection over Fujisawa be withdrawn.

O'Neil et al.

Claims 20, 38-40 and 42-44 stand rejected under 35 U.S.C. 102(b) as being anticipated by O'Neil et al., Synthetic Letters. The examiner cites O'Neil et al. (Syn. Lett.) as teaching the compounds, compositions and methods of use of the compounds of Formula III where R_6 is hydrogen, chloro or iodo, R_7 is hydrogen or chloro, R_8 is hydrogen, chloro, CH_2-CH, iodo or bromo and R_9 is hydrogen. Applicants have amended claim 20 to require that one of R_6, R_7, R_8 or R_9 is NH_2. O'Neil et al. (Syn. Lett.) does not teach or suggest the compounds of Formula III wherein one of R_6, R_7, R_8 or R_9 is NH_2. Applicants, therefore, submit that these claims, as amended, are allowable over the disclosure in O'Neil et al. (Syn. Lett.) and request that the rejection over O'Neil et al. (Syn. Lett.) be withdrawn.

Umezawa et al.

Claims 1, 11 and 38-44 stand rejected under 35 U.S.C. 102(b) as being anticipated by Umezawa et al. (JP 53-82792). The examiner states that Umezawa et al. teaches the compounds, compositions and methods of use of the compounds of Formula Ia where R_6 and R_7 are hydrogen, R_8 is methyl, R_9 is hydroxy, -A-R_2 is -CH=CH–C(=O)-NH(CH_3). Applicants have amended claim 1 such that the compounds disclosed in Umezawa et al. are specifically excluded from the claim. Applicants submit that Umezawa et al. does not teach or suggest the compounds of the claimed invention as amended, and therefore, respectfully request that the rejection over Umezawa et al. be withdrawn.

Gregson et al.

Claims 13-19 and 38-45 stand rejected under 35 U.S.C. 102(a) as being anticipated by Gregson et al. (Chem. Commun). The examiner cites Gregson et al. as teaching the compounds, compositions and method of use of the compounds of Formula II where R_6 is hydrogen, R_7 is methoxy, R_8 forms the dimer through the bridge -O–$(CH_2)_3$–O-, R_9 is hydrogen, and R'_2 is CH_2.

Gregson et al. was published on May 7, 1999 (see attached papers). Applicants respectfully direct the examiner's attention to the priority documents filed with the international application. Because applicants' priority date is August 27, 1998, Gregson et al. is not §102(a) prior art, which requires the reference to be publicly known. Therefore, applicants respectfully request that this rejection be withdrawn.

O'Neil et al.

Claims 20, 25-27 and 38-44 stand rejected under 35 U.S.C. 102(a) as being anticipated by O'Neil et al. (Tet. Lett.). The examiner cites O'Neil et al. (Tet. Lett.) as teaching the compounds, compositions and methods of use of the compounds of Formula III where R_6 is hydrogen or chloro, R_7 is hydrogen or fluoro, R_8 is hydrogen, fluoro, chloro, thien-2-yl, furan-2-yl or phenyl and R_9 is hydrogen. Applicants have amended claim 20 to require that one of R_6, R_7, R_8 or R_9 is NH_2. O'Neil et al. (Tet. Lett.) does not teach or suggest the compounds of Formula III wherein one of R_6, R_7, R_8 or R_9 is NH_2. Applicants, therefore, submit that these claims, as amended, are allowable over the disclosure in O'Neil et al. (Tet. Lett.) and request that the rejection over O'Neil et al. (Tet Lett.) be withdrawn.

Guiotto et al.

Claims 20, 25-27 and 38-44 stand rejected under 35 U.S.C. 102(a) as being anticipated by Guiotto et al. (Bioorg. & Med. Chem. Lett.). The examiner states that Guiotto teaches the compounds, compositions and method of use of the compounds of Formula III where R_6 is hydrogen, R_7 is phenyl, 4-methylphenyl, 4-fluorophenyl, 3-nitrophenyl, 2-methoxyphenyl or 4-methoxyphenyl, R_8 is hydrogen, and R_9 is hydrogen.

Guiotto et al. was published on November 3, 1998 (see attached papers). Applicants respectfully direct the examiner's attention to the priority documents filed with the international application. Because applicants' priority date is August 27, 1998, Guiotto et al. is not §102(a) prior art, which requires the reference to be publicly known. Therefore, applicants respectfully request that the rejection be withdrawn.

Thurston et al.

Claims 1, 7, 11, 20, 22 and 38-44 stand rejected under 35 U.S.C. 102(a) as being anticipated by Thurston et al. (J. Med. Chem.). The examiner states that Thurston et al. teaches the compounds, compositions and method of use of the compounds of Formula Ia and III where R_6 is hydrogen, R_7 is sugar-O-, hydroxy, or methoxy, R_8 is hydroxy, methoxy, $-O-CH_2-CH_2-COOCH_3$, $-O-(CH_2)_3-OCH_2Ph$ or $-NH-COOCH_2Ph$ and R_7 and R_8 together form a $-O-(CH_2)_2-O-$ or $-O-CH_2-O-$ moiety, and R_9 is hydrogen or methoxy, and $-A-R_2$ is $-CH=CH-CH_3$.

Thurston et al. (J. Med. Chem.) was published on June 3, 1999 in paper and May 18, 1999 on the web (see attached papers). Applicants respectfully direct the examiner's attention to the priority documents filed with the international application. Because applicants' priority date is August 27, 1998, Thurston et al. (J. Med. Chem.)

is not §102(a) prior art, which requires the reference to be publicly known. Therefore, applicants respectfully request that this rejection be withdrawn.

Thurston et al.

Claims 13-18, 20, 28 and 38-44 stand rejected under 35 U.S.C. 102(b) as being anticipated by Thurston et al. (Chem. Rev.). The examiner states that Thurston et al. (Chem. Rev.) teaches the compounds, compositions and method of use of the compounds of formula II and III where R_6 is hydrogen, R_7 is hydrogen or methoxy, R_8 is chloro, benzyloxy or 4-nitrobenzyloxy or R_7 and R_8 together form a $-O-CH_2-O-$ moiety, R_9 is hydrogen and R'_2 is $=CH=CH_3$. Applicants have amended claim 13 to require that the compound be a dimer and claim 20 to require that at least one of R_6, R_7, R_8 and R_9 be NH_2. Applicants respectfully submit that Thurston et al. (Chem. Rev.) does not teach or suggest the compounds of the claimed invention. Therefore, applicants respectfully request that the rejection over Thurston et al. (Chem. Rev.) be withdrawn.

Kunimoto et al.

Claims 1, 11 and 38-44 stand rejected under 35 U.S.C. 102(b) as being anticipated by Kunimoto et al. (J. Antibiotics). The examiner cites Kunimoto et al. as teaching the compounds, compositions and method of use of the compounds of Formula Ia where R_6 is hydrogen, R_7 is hydrogen, R_8 is methyl, R_9 is hydroxy and -A-R_2 is $-CH=CH-C(=O)-NHCH_3$. Applicants have amended claim 1 to specifically exclude the compounds disclosed in Kunimoto et al. Kunimoto et al. neither teaches nor suggests the compounds of the claimed invention. Applicants, therefore, respectfully request that the rejection over Kunimoto et al. be withdrawn.

Langley et al.

Claims 13-18 and 38-44 stand rejected under 35 U.S.C. 102(b) as being anticipated by Langley et al. (J. Org. Chem.). The examiner states that Langley et al. teaches the compounds, compositions and method of use of the compounds of Formula II where R_6 is hydrogen, R_7 is methoxy, R_8 is benzyloxy, R_9 is hydrogen and R'_2 is $=CH-CH_3$. Applicants have amended claim 13 so that R'_2 cannot be $=CHCH_3$. As a result, Langley et al. does not teach or suggest the claimed invention. As such, applicants request that the rejection over Langley et al. be withdrawn.

Baraldi et al.

Claims 20, 38-44 and 42-44 are rejected under 35 U.S.C. 102(a) as being anticipated by Baraldi et al. (Bioorg. & Med. Chem. Lett.). The examiner cites Baraldi as teaching the compounds, compositions and method of use of the compounds of formula III where R_6 is hydrogen, R_7 is methoxy, R_8 is $-O-(CH_2)_2-C(=O)-OCH_3$ and R_9 is hydrogen.

Baraldi et al. was published on November 3, 1998 (see attached papers). Applicants respectfully direct the examiner's attention to the priority documents filed with the international application. Because applicants' priority date is August 27, 1998, Baraldi et al. is not §102(a) prior art, which requires the reference to be publicly known. Therefore applicants respectfully request that this rejection be withdrawn.

Claim Rejections Under §103

Takanabe et al.

Claims 20 and 38-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Takanabe et al. (U.S. Patent No. 4,185,016). The examiner cites Takanabe as disclosing compounds of Formula II where in R_8 is a hydrogen atom, alkyl group, hydroxy group or alkoxy group and R_9 is a hydroxy or acyloxy group.

The examiner asserts that the "[c]ompounds of the instant invention are generically embraced by [Takanabe et al.] in view of the interchangeability of the R_1 and R_3 substituents of the tricyclic ung system. Thus, one of ordinary skill in the art at the time the invention was made would have been motivated to select, for example, hydroxy or ethoxy for R_1 as well as other possibilities from the generically disclosed alternatives of the reference and in so doing obtain the instant compounds in view of the equivalency teachings outlined above." (Official Action at 12-13.)

Applicants respectfully disagree with the examiner's view of Takanabe et al. for several reasons. First, applicants dispute the alleged interchangeability of the R_1 and R_3 substituents. If these substituents were truly interchangeable, they would not have been defined differently in Takanabe et al. Second, Takanabe et al. does not teach the amino substituent in amended claim 20 and does not give any indication that such a substituent would be beneficial. Moreover, the biological data presented in the present application, at page 206-207, Example 7, shows improved activity with the amino substituent. Compound 151, which contains an amino substituent at R_8 is more active than compounds 136, 138 and 96, which contain a phenyl subsituent at R_7, a 4'-methoxyphenyl substituent at R_7, and a methoxy substituent at R_7, respectively. Applicants respectfully submit that Takanabe et al. does not teach or suggest the claimed invention, and therefore, submit that claims 20 and 38-44 are not obvious in view of Takanabe et al. Applicants request that the rejection over Takanabe et al. be withdrawn.

Arima et al.

Claims 13-15, 17 and 38-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Arima et al. (GB 1 299 198). The examiner cites Arima et al. as disclosing compounds of Formula V. The examiner asserts that "[c]ompounds of the

instant invention are generically embraced by [Arima et al.] in view of the interchangeability of the R_1 substituent of the tricyclic ring system. Thus, one of ordinary skill in the art at the time the invention was made would have been motivated to select, for example, acetyl or benzoyl for R_1 as well as other possibilities from the generically disclosed alternatives of the reference and in so doing obtain the instant compounds in view of the equivalency teachings outlined above." (Official Action at 13.)

Applicants respectfully submit that there is no teaching in Arima et al. to change the nature of the R'_2 substituent. In fact, Arima et al. teaches that the =CH-CH_3 group is essential, as no other substituents are given. As amended, claim 13 requires that R'_2 be O. Therefore, applicants respectfully submit that the claimed invention is nonobvious in view of the disclosure in Arima et al., and request that this rejection be withdrawn.

Fujisawa Pharmaceutical Company

Claims 13-15, 17 and 38-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Fujisawa Pharmaceutical Company (JP 57-131791). The examiner cites Fujisawa as disclosing compounds of Formula I. The examiner asserts "[c]ompounds of the instant invention are generically embraced by [Fujisawa] in view of the interchangeability of the R^1 and R^2 substituents of the tricyclic ring system. Thus, one of ordinary skill in the art at the time the invention was made would have been motivated to select, for example, ethoxy or i-propoxy for R^2 as well as other possibilities from the generically disclosed alternatives of the reference and in so doing obtain the instant compounds in view of the equivalency teachings outline above." (Official Action at 13-14.)

Applicants have amended claims 13 to cover compounds of Formula II which are dimers. Fujisawa discloses only monomeric compounds. Therefore, applicants respectfully submit that Fujisawa does not teach or suggest the claimed invention and request that this rejection be withdrawn.

Langlois et al.

Claims 20-23 and 38-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Langlois et al. (FR 2 256 683). The examiner cites Langlois et al. '683 as disclosing some compounds encompassed by Formulae I and III. The examiner asserts that "[c]ompounds of the instant invention are generically embraced by [Langlois et al. '683] in view of the interchangeability of R_1, R_2, R_3, R_4, R_5, R_6 and R_7 substituents of the tricyclic ring system. Thus, one of ordinary skill in the art at the time the invention was made would have been motivated to select, for example, OCH_2O as well as other possibilities from the generically disclosed alternatives of the reference and in so doing obtain the instant compounds in view of the equivalency teachings outlined above." (Official Action at 14).

Applicants respectfully disagree with the examiner on the interchangeability of the disclosed substituents. Applicants submit that the examiner is using improper hindsight to reconstruct the present invention from the disclosure of Langlois et al. '683. Absent a teaching or suggestion in the reference to combine the substituents as claimed, the present invention is not obvious. Applicants submit that the substituents of Langlois et al. '683 are not interchangeable. The seven substituents are defined separately. As a result, one of ordinary skill in the art would not view them as interchangeable.

Nor is there any disclosure of the claimed amino substituent. One of ordinary skill in the art would not be led to use an amino substituent based on the disclosure of

Langlois et al.'683. Applicants respectfully submit that the present invention is not obvious in view of Langlois '683, and therefore, request that this rejection be withdrawn.

Langlois et al.

Claims 1, 7-11 and 38-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Langlois et al. (W092/19620). The examiner cites Langlois et al. '620 as disclosing compounds of Formula I. The examiner asserts that "[c]ompounds of the instant invention are generically embraced by [Langlois et al. '620] in view of the interchangeability of the R_1, R_2, R_3, R_4, x, y, z and n substituents of the tricyclic ring system. Thus, one of ordinary skill in the art at the time the invention was made would have been motivated to select, for example, ethoxy or i-propoxy for R^2 as well as other possibilities from the generically disclosed alternatives of the reference and in so doing obtain the instant compounds in view of the equivalency teachings outlined above." (Official Action at 15.)

Again, applicants respectfully submit that the substituents are not equivalent or interchangeable. If this were the case, Langlois et al. '620 would not contain five different definitions for the seven substituents. Thus, Langlois et al. '620 does not teach that these substituents are interchangeable. Nor does Langlois et al. '620 teach or suggest that R_1, R_2, R_3 or R_4 could be an amino substituent. Therefore, the present invention is not obvious in view of Langlois et al. '620 and applicants respectfully request that the rejection over Langlois et al. '620 be withdrawn.

Thurston et al.

Claims 20-24, 28 and 38-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thurston et al. (WO 93/18045). The examiner cites Thurston et al. '045 as disclosing compounds of Formula I. The examiner asserts that

"[c]compounds of the instant invention are generically embraced by [Thurston et al. '045] in view of the interchangeability of the R and X substituents plus the additional substituents in one or more of the 1, 2, 3, 6, 7, 9 and 11-positions of the tricyclic ring system. Thus, one of ordinary skill in the art at the time the invention was made would have been motivated to select, for example, OCH_2CH_3 or hydroxy as well as other possibilities from the generically disclosed alternatives of the reference and in so doing obtain the instant compounds in view of the equivalency teachings outlined above." (Official Action at 16.)

Applicants submit that the substituents as disclosed in Thurston et al. '045 are not interchangeable. Thurston et al. teaches that the substitutents are not interchangeable in that it separately defines the different substituents. Nor does Thurston et al. '045 teach or suggest that one of R_6, R_7, R_8 or R_9 may be NH_2. Therefore, applicants respectfully submit that the present invention is not obvious in view of Thurston et al. '045, and request that this rejection be withdrawn.

Provisional Obviousness-Type Double Patenting Rejection

Claims 1-45 stand provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over the claims of copending U.S. Application Serial No. 09/763,813 ("the '813 application") copending U.S. Application Serial No. 09/763,768 ("the '768 application") and U.S. copending application Serial No. 09/763,814 ("the '814 application"). The examiner asserts that the conflicting claims are not patentably distinct from one another because the compounds of the instant invention are embraced by the library of the '813 application, the '768 application and the '814 application.

Applicants respectfully traverse the examiner's provisional rejection for several reasons. The examiner has not established a *prima facie* case of obviousness.

As discussed above, the examiner must show that any variation between the inventions claimed in the present application and those in the copending applications would have been obvious to one skilled in the art. To do so, the analysis must follow the analysis set forth in *Graham v. John Deere Co.*, 383 U.S. 1 (1966). That analysis is as follows:

(1) the scope and content of the prior art must be determined;

(2) the differences between the prior art and the claims must be ascertained;

(3) the level of ordinary skill in the pertinent art must be determined; and

(4) any evidence of secondary considerations must be evaluated.

Graham, 383 U.S. 1 (1966).

The examiner has provided no analysis as to the difference between the inventions claims by the conflicting claims compared to a claim in the instant application, and so to the reasons why a person of ordinary skill in the art would have concluded that the invention defined in the claims of the present invention is an obvious variant of the inventions defined in the claims of the copending applications.

Rather, the examiner has asserted nothing more than the bold conclusion that the compounds of the present invention are embraced by the libraries of the '814, '768 and '813 applications. Even if this were the case, a genus-species relationship is not a litmus test for resolving the question of obviousness-type double patenting.

Because the double patenting rejections are provisional as no claims in any of the applications have been patented, and as a *prima facie* case of obviousness has not been established, any consideration of a terminal disclaimer at this time is improper.

Claim Objections

Claims 6-12, 19, 25-28 and 32-45 were objected to under 37 C.F.R. § 1.75(c) as being in improper form because a multiple dependent claim must refer to the claims from which they depend in the alternative and a multiple dependent claim cannot depend on another multiple dependent claim. Applicants respectfully submit that the amendments to the claims obviate these objections, and therefore, request that the objections be withdrawn.

Conclusion

In view of the foregoing, applicants respectfully submit that the claims, as amended, are in condition for allowance. Applicants earnestly solicit a Notice of Allowance.

Respectfully submitted,

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Version With Markings

In the Specification:

Please replace the paragraph beginning at page 1, line 4 with the following:

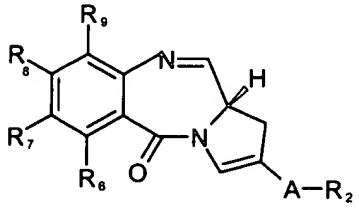
[Background to the invention] BACKGROUND TO THE INVENTION

Please replace the paragraph beginning at page 2, line 15 with the following

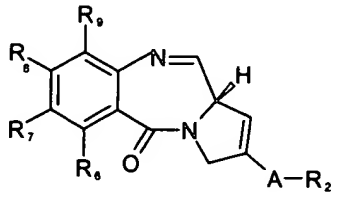
[Disclosure of the invention] DETAILED DESCRIPTION OF THE INVENTION

In the Claims:

1. (Once amended.) A compound of the formula IIa or IIb:



(Ia)



(Ib)

wherein:

A is CH_2, or a single bond;

R_2 is selected from: R, OH, OR, CO_2H, CO_2R, COH, COR, SO_2R, CN;

R_6, R_7 and R_9 are independently selected from H, R, OH, OR, halo, amino, NHR, nitro, Me_3Sn;

where R is a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group of up to 12 carbon atoms, whereof the alkyl group optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system, or an aryl group of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally containing one or more hetero atoms which may form part of, or be, a functional group;

and [R_8 is selected from H, R, OH, OR, halo, amino, NHR, nitro, Me_3Sn, where R is as defined above or] where the compound is a dimer with each monomer being the same or different and being of formula IIa or IIb, where the R_8 groups of the monomers form together a bridge having the formula -X-R^1-X- linking the monomers, where R^1 is an alkylene chain containing from 3 to 12 carbon atoms, which chain may be interrupted by one or more hetero-atoms and/or aromatic rings and may contain one or more carbon-carbon double or triple bonds, and each X is independently selected from O, S, or N[; or R_7 and R_8 together form a group -O-[])]$(CH_2)_p$-O-, where p is 1 or 2; [except that in a compound of formula IIa when A is a single bond, then R_2 is not CH=CH($CONH_2$) or CH=CH($CONMe_2$)] with the proviso that when A is a single bond, then R_2 is not CH=CR^AR^B, where R^A and R^B are independently selected from H, R^C, COR^C, $CONH_2$, $CONHR^C$, $CONR^C_2$, cyano or phosphonate, where R^C is an unsubstituted alkyl group having 1 to 4 carbon atoms.

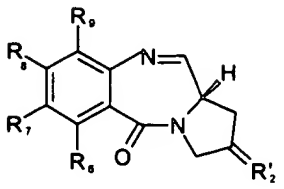
3. (Once amended.) A compound according to [either] claim 1 [or claim 2], wherein A is CH_2.

6. (Once amended.) A compound according to claim 1 [or claim 3], wherein A is a single bond, and R_2 is an aryl group, or an alkyl or alkaryl group which contains at least one double bond which forms part of a conjugated system with [the] a double bond of [the C-ring] a pyrrolobenzodiazepine.

7. (Once amended.) A compound according to claim 1 [any one of the preceding claims] wherein R_6, R_7 and R_9 and, unless the compound is a dimer, R_8 are independently selected from H and OR.

12. (Once amended.) A compound according to claim 1 [any one of the preceding claims] which is a dimer, wherein the dimer bridge is of the formula -O-$(CH_2)_{[p]q}$-O-, where [p] q is from [1]3 to 12.

13. (Once amended.) A compound of formula III:



(II)

wherein:

R'_2 is selected from: O [, CHR''_2, where R''_2 is selected from H, R, CO_2R, COR, CHO, CO_2H, halo];

R_6, R_7 and R_9 are independently selected from H, R, OH, OR, halo, amino, NHR, nitro, Me_3Sn;

where R is a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group of up to 12 carbon atoms, whereof the alkyl group optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system, or an aryl group of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally containing one or more hetero atoms which may from part of, or be, a functional group;

and [R_8 is selected from H, R, OH, OR, halo, amino, NHR, nitro, Me_3Sn, where R is as defined above or] where the compound is a dimer with each monomer being the same or different and being of formula III, where the R_8 groups of the monomers form together a bridge having the formula -X-R^1-X- linking the monomers, where R^1 is an alkylene chain containing from 3 to 12 carbon atoms, which chain may be interrupted by one or more hetero-atoms and/or aromatic rings and may contain one or more carbon-carbon double or triple bonds, and each X is independently selected from O, S, or N[; or R_7 and R_8 together form a group -O-$(CH_2)_p$-O-, where p is 1 or 2;

except that:

(i) when $R=_2$ is CH-Et, and R_6, R_8 and R_9 are H, R_7 is not sibirosamine pyranoside; and

(ii) when $R=_2$ is CH-Me, and R_6 and R_9 are H, R_7 and R_8 are not both H or both OMe, or OMe and OH respectively].

15. (Once amended.) A compound according to [either] claim 13 [or claim 14], wherein R_6, R_7 and R_9 [and, unless the compound is a dimer, R_8] are independently selected from H, OR or a halogen atom.

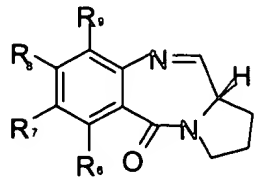
16. (Once amended.) A compound according to claim 15, wherein R_6, R_7 and R_9 [and, unless the compound is a dimer, R_8] are independently selected from H, OMe, [and] OCH_2Ph, and I.

17. (Once amended.) A compound according to claim 15, wherein R_7 [and, unless the compound is a dimer, R_8 are independently] is OR or a halogen and R_6 and R_9 are H.

18. (Once amended.) A compound according to claim 17, wherein R_7 [and, unless the compound is a dimer, R_8 are independently] is selected from OMe, OCH_2Ph or I.

19. (Once amended.) A compound according to claim 13 [any one of claims 13 to 18 which is a dimer], wherein the dimer bridge is of the formula $-O-(CH_2)_{[p]q}-O-$, where [p] q is from [1]3 to 12.

20. (Once amended.) A compound of the formula III:



(III)

wherein:

R_6, R_7 and R_9 are independently selected from H, R, OH, OR, halo, amino, NHR, nitro, Me_3Sn;

where R is a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group of up to 12 carbon atoms, whereof the alkyl group optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system, or an aryl group of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally containing one or more hetero atoms which may from part of, or be, a functional group;

and R_8 is selected from H, R, OH, OR, halo, amino, NHR, nitro, Me_3Sn, where R is as defined above or the compound is a dimer with each monomer being the same or different and being of formula III, where the R_8 groups of the monomers form together a bridge having the formula -X-R^1-X- linking the monomers, where R^1 is an alkylene chain containing from 3 to 12 carbon atoms, which chain may be interrupted by one or more hetero-atoms and/or aromatic rings and may contain one or more carbon-carbon double or triple bonds, and each X is independently selected from O, S, or N; or R_7 and R_8 together form a group -O-[)][$(CH_2)_p$-O-, where p is 1 or 2;

wherein at least one of R_6, R_7, R_8 and R_9 <u>is NH_2</u> [are not H;

except that:

(i) when R_6 and R_9 are H, R_7 and R_8 are not both OMe, OMe and OBn respectively, or OMe and OH respectively;

(ii) when R_6 and R_7 are H, R_8 and R_9 are not Me and OH respectively;

(iii) when three of R_6, R_7, R_8 and R_9 are H, the other is not Me;

(iv) when R_6, R_7, and R_8 are H, R_9 is not OMe;

(v) when R_6, R_8 and R_9 are H, R_7 is not OMe; and

(vi) when R_6, and R_9 are H and R_7 is OMe, the compound is not a dimer].

25. (Once amended.) A compound according to claim 20 [, claim 21 or claim 24], wherein at least one of R_6, R_7, R_8 and R_9 is an aryl group [, preferably] of up to 12 carbon atoms, which is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally contains one or more hetero atoms which may from part of, or be, a functional group.

28. (Once amended.) A compound according to [any one of] claim[s] 20 [to 27] where the compound is a dimer, wherein the dimer bridge is of the formula -O-$(CH_2)_{[p]q}$-O-, where [p] q is from [1]3 to 12.

31. (Once amended.) A compound according to [either] claim 29 [or 30], wherein R_7 is an electron [withdrawing] donating group.

32. (Once amended.) A compound according to [any one of] claim[s] 29 [to 31], wherein R_6 and R_9 are selected from H and OR.

34. (Once amended.) A compound according to [any one of] claim[s] 30 [to 33], wherein n is 1 to 3.

35. (Once amended.) A compound according to claim 1, claim 13, claim 20 or claim 29 [any one of the preceding claims] wherein R is selected from a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group of up to 12 carbon atoms, or an aryl group of up to 12 carbon atoms, optionally substituted by one or more halo, hydroxy, amino, or nitro groups.

38. (Once amended.) A method of treating a condition which can be treated by regulation of gene expression comprising administering [The use of] a compound according to claim 1, claim 13, claim 20 or claim 29 [any one of the preceding claims in a method of therapy] to a patient in need of such treatment.

40. (Once amended.) A method of treating a gene-based disease comprising administering an effective amount of [The use of] a compound according to claim 1, claim 13, claim 20 or claim 29 [any one of claims 1 to 37 to prepare a medicament for the treatment of a gene-based disease] to a patient in need of such treatment.

41. (Once amended.) A method of treating a viral, parasitic or bacterial infection comprising administering an effective amount of [The use of] a compound according to claim 1, claim 13, claim 20 or claim 29 [any one of claims 1 to 37 to prepare a medicament for the treatment of a viral, parasitic or bacterial infection] to a patient in need of such treatment.

43. (Once amended.) A method of treating a cisplatin-refractory disease comprising administering an effective amount of [The use of] a compound according to claim 1, claim 13, claim 20 or claim 29 [any one of claims 1 to 37 to prepare a medicament for the treatment of a cisplatin-refractory disease] to a patient in need of such treatment.

44. (Once amended.) A method of inhibiting the growth of cisplatin-[refactory]refractory cells which method comprises treating said cells with a compound according to [any one of claims 1 to 37] claim 1, claim 13, claim 20 or claim 29.

45. (Once amended.) A method according to claim 44 wherein said compound is [SJG-136] 1,1'-[[(Propane-1,3-diyl)dioxy]bis(11a*S*)-7-methoxy-2-methylidene-1,2,3,11a-tetro-hydro-*5H*-pyrrolo [2,1-c] [1,4] denzodiazepin-5-one].

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Articles

Highly Potent Cyclic Disulfide Antagonists of Somatostatin

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Received November 5, 1998

The search for synthetic analogues of somatostatin (SRIF) which exhibit selective affinities for the five known receptor subtypes (sst_{1-5}) has generated a large number of potent agonist analogues. Many of these agonists display good subtype selectivities and affinities for the subtypes 2, 3, and 5, with very few selective for sst_1 or sst_4. Until the recent report by Bass and co-workers (*Mol. Pharmacol.* 1996, *50*, 709−715; erratum *Mol. Pharmacol.* 1997, *51*, 170), no true antagonists of somatostatin had been discovered, let alone any displaying differential receptor subtype selectivity. In this present study, we further explore the effect of this putative L,^{5}D^6 antagonist motif on somatostatin octapeptide analogues with a cyclic hexapeptide core. The most potent antagonist found to date is H-Cpa-cyclo[DCys-Tyr-DTrp-Lys-Thr-Cys]-Nal-NH_2, PRL-2970 (21), which has an IC_{50} of 1.1 nM in a rat pituitary growth hormone in vitro antagonist assay versus SRIF (1 nM). This analogue bound to cloned human somatostatin subtype 2 receptors with a K_i of 26 nM. The highest $hsst_2$ affinity analogue was H-Cpa-cyclo-[DCys-Pal-DTrp-Lys-Tle-Cys]-Nal-NH_2, PRL-2915 (15), with a K_i of 12 nM (IC_{50} = 1.8 nM). This analogue was also selective for $hsst_2$ over $hsst_3$ and $hsst_5$ by factors of 8 and 40, respectively, and had no agonist activity when tested alone at concentrations up to 10 μM. Regression analysis of the binding affinities versus the observed antagonist potencies revealed high correlations for $hsst_2$ (r = 0.65) and $hsst_3$ (r = 0.52) with a less significant correlation to $hsst_5$ (r = 0.40). This is quite different from the somatostatin agonist analogues which show a highly significant correlation to $hsst_2$ (r > 0.9). Receptor-selective somatostatin antagonists should provide valuable tools for characterizing the many important physiological functions of this neuropeptide.

Introduction

The tetradecapeptide somatostatin (SRIF)[1] is a potent regulator of multiple biological functions. Although SRIF was originally isolated from mammalian hypothalamii and characterized as a potent physiological inhibitor of growth hormone (GH) secretion from the anterior pituitary, SRIF also inhibits the pancreatic secretion of glucagon and insulin[2] and the secretion of gastrin from the gut.[3] Additionally, SRIF acts as a neurotransmitter or neuromodulator in the central nervous system and peripheral tissues where it modulates neuronal firing,[4,5] release of other neurotransmitters,[6,7] motor activity,[8] and cognitive processes.[9] These biological effects of SRIF, mostly inhibitory in nature, are elicited through a series of G protein-coupled, transmembrane receptors, of which five different recep-

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ect of A-Ring Modifications on the DNA-Binding Behavior and Cytotoxicity Pyrrolo[2,1-*c*][1,4]benzodiazepines†

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Gene Targeted Drug Design Research Group, School of Pharmacy and Biomedical Sciences, University of Portsmouth, Mibcel's Building, White Swan Road, Portsmouth, Hants P01 2DT, U.K., School of Chemical and Life Sciences, ersity of Greenwich, Wellington Street, Woolwich, London SE18 6PF, U.K., University of Ferrara, Via Fossato di 17/19, I-44100 Ferrara, Italy, CRC Centre for Cancer Therapeutics, Institute for Cancer Research, Royal Cancer Hospital, Block E, Clifton Avenue, Sutton, Surrey SM2 5PX, U.K., and Centre d'Etudes et de Recherche sur ment de Normandie (CERMN), U.F.R. des Sciences Pharmaceutiques 1, rue Vaubenard 14032, Caen, France

November 12, 1998

Several A-ring-modified analogues of the DNA-binding antitumor agent DC-81 (**5**) have been synthesized in order to study structure–reactivity/cytotoxicity relationships. For two molecules (**23** and **30**) the modifications required the addition of a fourth ring to give the novel dioxolo-[4,5-*h*]- and dioxano[5,6-*h*]pyrrolo[2,1-*c*][1,4]benzodiazepin-11-one (PBD) ring systems, respectively. Another three analogues (**34**, **38**, and **48**) have the native benzenoid A-ring replaced with pyridine, diazine, or pyrimidine rings to give the novel pyrrolo[2,1-*c*][1,4]pyridodiazepine, pyrrolo[2,1-*c*][1,4]diazinodiazepine, and pyrrolo[2,1-*c*][1,4]pyrimidinodiazepine systems, respectively. The other new analogues (**16a,b**) have extended chains at the C8-position of the DC-81 structure. During the synthesis of these compounds, a novel tin-mediated regiospecific cleavage reaction of the dioxole intermediate **18** was discovered, leading to the previously unknown *iso*-DC-81 (**20**). In addition, an unusual simultaneous nitration–oxidation reaction of 4-(3-hydroxypropoxy)-3-methoxybenzoic acid (**8**) was found to produce 3-(4-carboxy-2-methoxy-5-nitrophenoxy)propanoic acid (**9**), a key intermediate, in high yield. In general, the results of cytotoxicity and DNA-binding studies indicated that none of the changes made to the A-ring of the PBD system significantly improved either binding affinity or cytotoxicity in comparison to DC-81. This result suggests that the superior potency of natural products such as anthramycin (**1**), tomaymycin (**2**), and sibiromycin (**3**) is due entirely to differences in C-ring structure, and in particular *exo* or *endo* unsaturation at the C2-position and C2-substituents containing unsaturation. This study also provided information regarding the influence of A-ring substitution pattern on the relative stability of the interconvertible N10–C11 carbinolamine, carbinolamine methyl ether, and imine forms of PBDs.

troduction

Currently there is interest in discovering and developing small molecules capable of binding to DNA in a highly sequence-selective manner,[1] as the ability to target and then down-regulate individual genes has potential in the therapy of genetic based diseases (e.g., cancer[2]), diagnostics, functional genomics, and target validation. The pyrrolo[2,1-*c*][1,4]benzodiazepine antitumor antibiotics (PBDs, Figure 1) are a well-known class of sequence-selective DNA-binding agents derived from *Streptomyces* species.[3] Their precise mode of interaction with DNA is unique and has been extensively studied; they bind within the minor groove of DNA, forming a covalent aminal bond between the C11-position of the central B-ring and the N2-amino group of a guanine base. A series of footprinting,[4,5] fluorescence,[6] molecular modeling,[7] and NMR[8] studies have shown that the molecules have a preferred selectivity for Pu-G-Pu (Pu, purine; G, alkylated guanine) sequences and can orientate with their A-rings pointing either toward the 3′- or 5′-end of the covalently bound strand.[3] Furthermore, the stereochemistry at the C11-position of the PBD can be in either the *R* or *S* configuration, thus giving rise to a total of four possible isomers for each DNA adduct.[3] Many members of the PBD family such as anthramycin (**1**) and tomaymycin (**2**) (Figure 1) have significant in vitro cytotoxicity, and some compounds, such as anthramycin and neothramycin (e.g., **4**), have reached various stages of clinical trials but have not progressed due to problems including cardiotoxicity or lack of efficacy, respectively.[9] However, recently there have been a number of attempts to enhance sequence selectivity and antitumor potency by synthesizing C7-[10] or C8-linked[11] PBD dimers, such as

† Abbreviations: EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole; PBD, pyrrolo[2,1-*c*][1,4]benzodiazepine.

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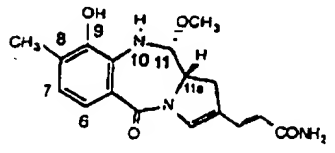
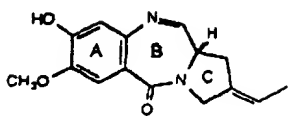
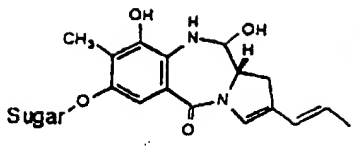
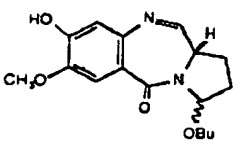
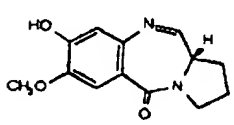
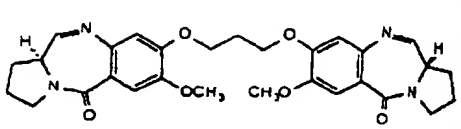

anthramycin methyl ether (1)


tomaymycin (2)


sibiromycin (3)


neothramycin A & B C3-*O*-butyl ethers (4)


DC-81 (5)


DSB-120 (6)

Figur 1. Structures of anthramycin (1), tomaymycin (2), sibiromycin (3), neothramycins A and B (4), DC-81 (5), and DSB-120 (6).

potent irreversible interstrand cross-linking agents known to date, capable of covalently linking two complementary DNA stands through interaction with guanine bases in the minor groove.[11,12] [**Note added in proof:** SJG-136, an analogue of DSB-120, has recently been reported to be significantly more cytotoxic than DSB-120 across a number of cell lines and to be >10-fold more potent at stabilizing DNA on a concentration basis according to thermal denaturation experiments (Gregson, S. J.; Howard, P. W.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. Synthesis of a Novel C2/C2′-*exo* Unsaturated Pyrrolobenzodiazepine Cross-linking Agent with Remarkable DNA Binding Affinity and Cytotoxicity. *J. Chem. Soc., Chem. Commun.* **1999**, *9*, 797–798).] It is also significantly more cytotoxic than the corresponding monomer DC-81. It has been demonstrated that the pyrrolobenzodiazepines can interfere with endonuclease activity[13] and can inhibit in vitro transcription in a highly sequence-selective manner.[14]

There has been a considerable effort by a number of groups to develop new synthetic routes to the PBD ring system, and these have been reviewed.[15] However, due mainly to the difficulties associated with synthesizing useful quantities of PBD analogues, little work regarding structure–activity relationships (SAR) has been published. In 1983, Hurley and Thurston tried to rationalize differences in biological activity of the known natural products using a CPK model of the DNA–PBD adduct.[16] Later, the same workers reported structure–binding and structure–cytotoxicity data for a limited series of A- and C-ring-modified synthetic analogues.[5] Doyle and co-workers have published the results of a study of bicyclic analogues of PBDs comprising only the A- and B-rings, demonstrating the importance of the presence of the C-ring for significant cytotoxicity.[17] More recently, Baraldi and co-workers have reported conversion of the aromatic A-ring of the PBD system into a pyrazole.[18] We report here the results of an investigation into the effect of modifying the A-ring complexion and substitution pattern on both DNA-binding reactivity and cytotoxic potency. Anthramycin (1), tomaymycin (2), sibiromycin (3), the C3-*O*-butyl neothramycins (4), DC-81 (5), the C8-benzyl (**52**) and C8-benzoyloxycarbonylamino (**53**)[19] DC-81 analogues, the A/C-ring-unsubstituted saturated analogue **50**,[19] and the pyrazine analogues[18] **49** and **51** were included in the present study for comparison.

Synthesis

C8-Substituted PBDs (16a,b) (Scheme 1). The C8-substituted DC-81 analogues **16a,b** (Scheme 1) were synthesized starting from vanillic acid (**7**) using the thioacetal route of Langley and Thurston.[20] Treatment of **7** with 3-bromopropanol in refluxing aqueous NaOH for 5 h afforded the alcohol **8** in >80% yield.[21] Anhydrous conditions with KOH in absolute EtOH were found to be less efficient, giving lower yields and requiring longer reaction times. Reaction of **8** with 70% aqueous HNO_3 at 0 °C[22] effected concurrent nitration at the 6-position and oxidation of the aliphatic alcohol to give the dicarboxylic acid **9** as a single product in good yield (>70%). After selective methylation (>80%) of the aliphatic carboxyl group (MeOH/*p*-TsOH) to give **10**, coupling to (2*S*)-pyrrolidine-2-carboxaldehyde diethyl thioacetal (**13**), prepared in six steps from L-proline,[20] afforded the nitro thioacetal **14a**. Subsequent reduction to **15a** with $SnCl_2 \cdot 2H_2O$ in MeOH and cyclization using $HgCl_2/CaCO_3$ afforded the PBD **16a** in the N10–C11 imine form as a stable solid after several evaporations from dry $CHCl_3$. The benzyl ether analogue **16b** was similarly prepared from the known 4-(3-hydroxypropoxy)-2-nitrovanillic acid fragment **11**. After *O*-benzylation of **11** using NaH and $PhCH_2Br$ in THF to give **12**, coupling to **13** gave the resulting amide **14b**, which was reduced (**15b**) and cyclized (**16b**) under identical conditions to that described above.

***iso*-DC-81 (20) and Dioxolo[4,5-*h*]pyrrolo[2,1-*c*][1,4]benzodiazepin-11-one PBD (23) (Scheme 2).** The key precursor **19a** used in the synthesis of *iso*-DC-81 (**20**) was obtained unexpectedly[23] during the synthesis of the novel dioxolo[4,5-*h*]pyrrolo[2,1-*c*][1,4]benzodiazepin-11-one PBD (**23**). The previously reported 2-nitropiperonylic acid (**17a**)[24] was coupled to (2*S*)-pyrrolidine-2-carboxaldehyde diethyl thioacetal (**13**) to afford the nitro thioacetal **18**. On the basis of previous experience,[11,15,19,25,26,27] **18** was treated with $SnCl_2 \cdot 2H_2O$ in refluxing MeOH to reduce the aromatic nitro group. Surprisingly, in addition to the anticipated reduction of the nitro group, the dioxole ring opened in a completely regiospecific manner to afford **19a**.[23] The methylenedioxy proton signal at δ 6.2 in the ^{1}H NMR spectrum of **18** was replaced with a methoxy signal at δ 3.9 in the spectrum of **19a**. Comparison of the NMR data with those of the DC-81 precursor **19b**[27] indicated

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SYNTHESIS OF NOVEL C7-ARYL SUBSTITUTED PYRROLO[2,1-*c*][1,4]BENZODIAZEPINES (PBDs) *VIA* PRO-N10-TROC PROTECTION AND SUZUKI COUPLING

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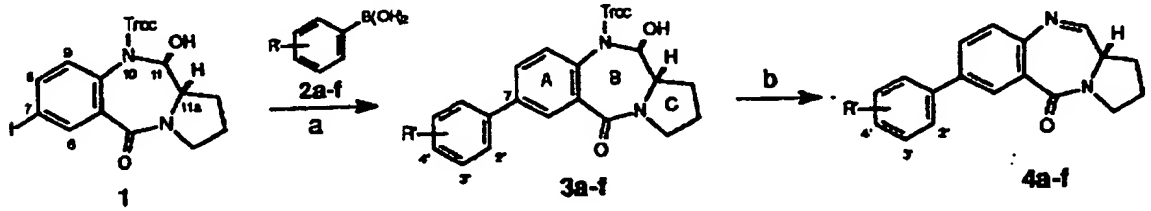
Abstract: Novel C7-aryl pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) have been synthesized via Suzuki coupling between a 7-Iodo N10-Troc-protected PBD carbinolamine and commercially available boronic acids.

The pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) antitumour antibiotics bond covalently to the N2 of guanines in the minor groove of DNA[1], inhibiting the interaction of enzymes with DNA[2] including the process of transcription[3]. There is interest in developing this group of compounds as gene targeting vectors and as antitumour and diagnostic agents[4]. We have recently applied a novel and concise synthetic approach to the PBD ring system based on the use of a pro-N10-2,2,2-trichloroethyl carbamate (Troc) protecting group[5,6] which can be removed easily under mild conditions. The ability to synthesize the 7-iodo N10-Troc-protected intermediate (**1**, Scheme 1) prompted us to investigate methods of C-C bond formation at the C7-position. PBDs of this type are of interest because of the *para* relationship of the C7-substituent to the N10 nitrogen atom of the DNA-interactive N10-C11 imine moiety. Molecular modeling studies have suggested[4] that although C7-substituents point out of the minor groove and do not appear to have a significant steric effect on DNA interaction, they can influence the electronic characteristics of the imine, thus affecting its electrophilicity and ability to interact with DNA.

Suzuki coupling[7] is based on the palladium-catalyzed reaction of organoboron compounds with aryl iodides and appeared to be an attractive means to generate biphenyls in the PBD system due to the mild conditions involved (Palladium catalyst, Na_2CO_3, refluxing benzene/water) and the relative stability of organoboron compounds. Using the previously synthesized 7-iodo N10-Troc-protected PBD (**1**) and commercially available boronic acids as starting materials, the 7-aryl PBDs **4a-f** have been synthesized in yields of up to 93% (Scheme 1 and Table 1).



Scheme 1: a: $Pd(Ph_3P)_4$, Na_2CO_3, benzene, H_2O; b: 10% Cd/Pb couple, 1N NH_4OAc aq.

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BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY OF A PYRROLO [2,1-c][1,4]BENZODIAZEPINE (PBD)-DISTAMYCIN HYBRID

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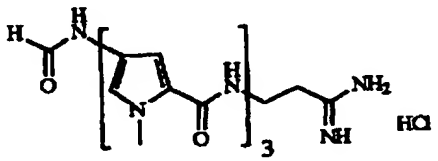
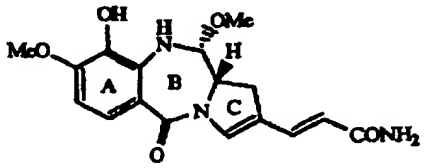
Abstract: We report the synthesis of a new hybrid **13** which is a combination of the naturally occurring antitumor agent distamycin A **1** and the pyrrolo[2,1-c][1,4]benzodiazepine **11**, related to the naturally occurring anthramycin **2**. The antitumor activity of the hybrid **13** was tested *in vitro* and compared to the natural product distamycin **1** and the PBD **11**.

Introduction

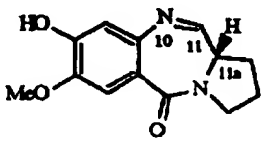
In the last few years, a growing interest has been shown in the development of DNA minor-groove binders acting as vehicles for the delivery of alkylating agents to DNA targets, and typical examples of this new class of compounds include alkylating derivatives of distamycin A **1**[1]. Distamycin A is a naturally occurring antiviral agent, isolated from cultures of *Streptomyces distallicus* [2,3]. Distamycin displays a high affinity for AT-rich sequences and binds reversibly, by noncovalent interactions, to the minor groove of double-helical B DNA. The pyrrolo[2,1-c][1,4]benzodiazepine (PBD) group[4], which includes the natural compounds anthramycin **2**[5] and DC-81 **3**, owes its DNA-interactive ability and resultant biological effects to a N10-C11 carbinolamine/imine moiety in the central B-ring which is capable of covalently binding to the C2-NH_2 of guanine residues in the minor groove of DNA. X ray and footprinting studies on covalent DNA-PBD adducts have shown a high sequence-specificity, for G-C rich DNA regions, in particular for X-G-X triplets (X=purine)[6].



1 Distamycin A



2 Anthramycin



3 DC-81

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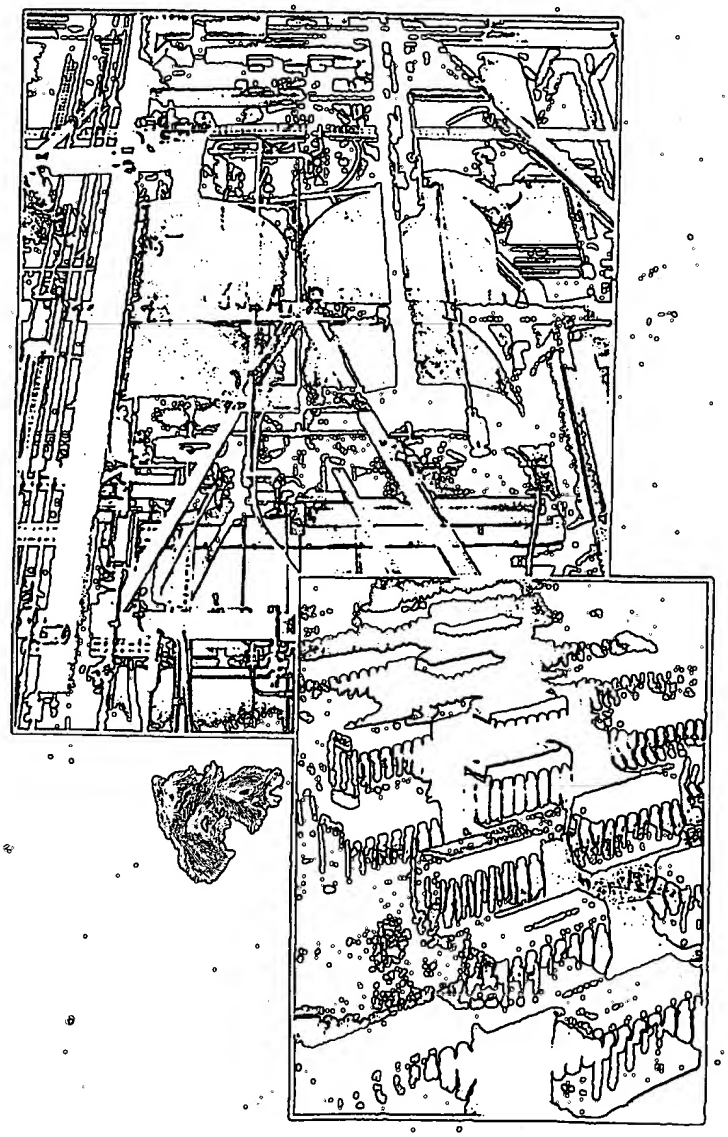

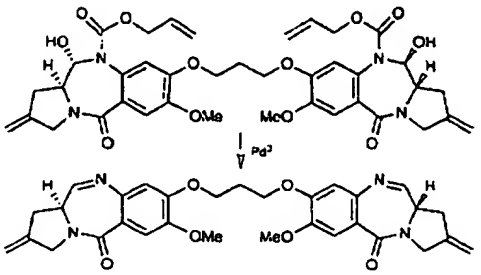

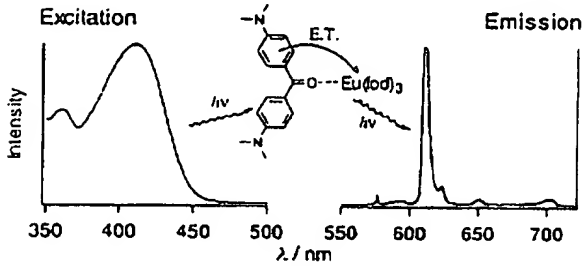

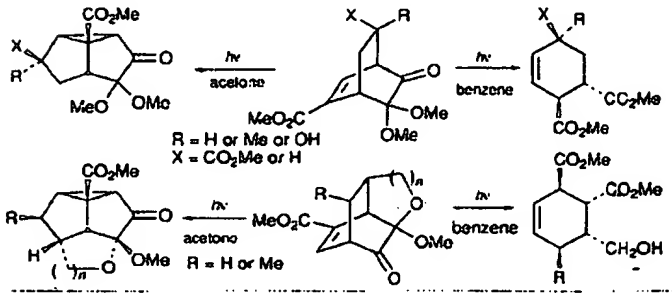

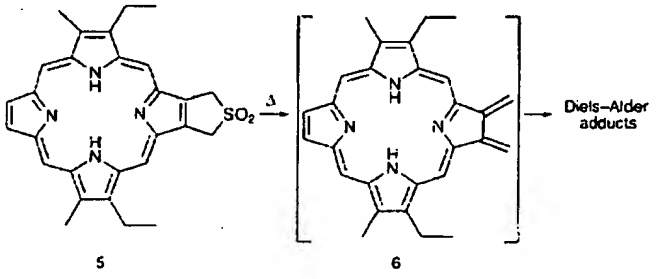

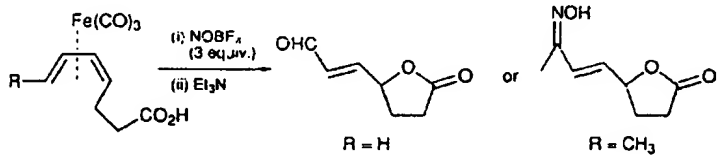

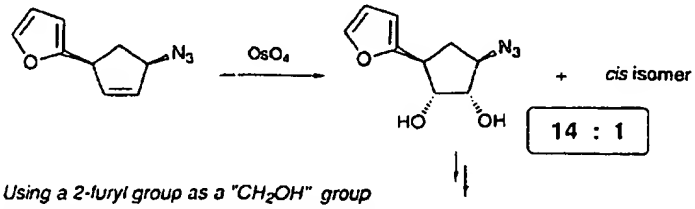

Synthesis of a novel C2/C2′-*exo* unsaturated pyrrolobenzodiazepine cross-linking agent with remarkable DNA binding affinity and cytotoxicity

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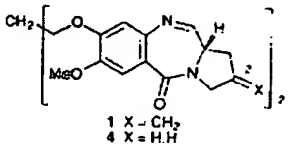
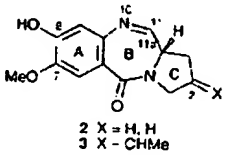
A C2/C2′-*exo* unsaturated pyrrolobenzodiazepine dimer 1 has been synthesised which is cytotoxic at the picomolar level and has remarkable covalent DNA binding affinity, raising the melting temperature of duplex-form calf thymus DNA by 34 °C after 18 h incubation.

There is presently interest in low molecular weight ligands that can interact with nucleic acids in a sequence-selective manner. Such agents have potential use in the validation of DNA sequences as potential therapeutic targets, in the therapy of genetic-based diseases (*e.g.* cancer[1,2]), and in the development of diagnostic agents. The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a family of antitumour antibiotics derived from various *Streptomyces* species that exert their biological activity by interacting with DNA in a sequence-selective fashion, forming a covalent bond between their electrophilic C11-position and the exocyclic C2-NH_2 group of a guanine base in the minor groove of DNA.[3] Recently, it has been demonstrated that PBDs can inhibit both endonuclease activity[4] and *in vitro* transcription[5] in a highly sequence-selective manner.

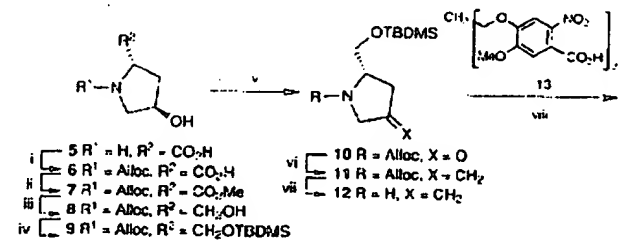
Although the parent PBDs span approximately three base pairs with a preference for purine–guanine–purine (*e.g.* AGA) sequences, a series of C-ring-unsubstituted C8-diyldioxy ether-linked PBD dimers have been synthesised (*e.g.* DSB-120 4) that

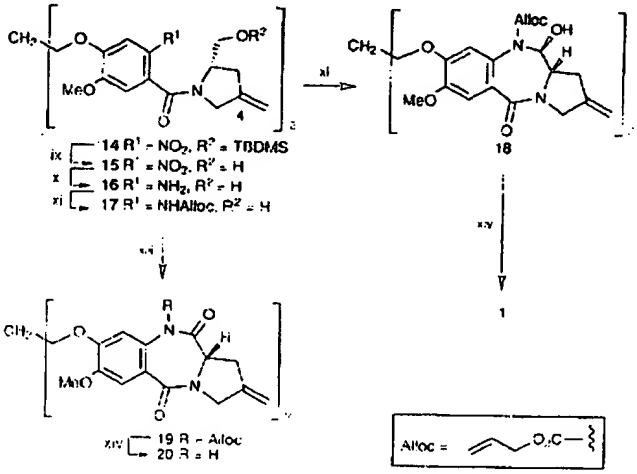




span approximately six base pairs of DNA and have enhanced sequence selectivity (*e.g.* purine-GATC-pyrimidine for DSB-120).[6,7] The sub-micromolar cytotoxicity of DSB-120 has been attributed to its ability to irreversibly cross-link DNA *via* guanine residues on opposite strands.[8] In an attempt to further extend base-pair span and recognition behaviour, we have investigated the inclusion of C2/C2′ substituents that should follow the contour of the host minor groove. Here, we report a novel synthesis of SJG-136 **1**, a C2/C2′*exo*-methylene analogue of DSB-120. This molecule has exquisite cytotoxicity in the picomolar region (*i.e.* IC_{50} = 0.000024 μM) in the cisplatin-resistant A2780cis human ovarian carcinoma cell line, some 9000-fold more potent than DSB-120 (IC_{50} = 0.21 μM). Furthermore, SJG-136 raises the melting temperature of calf thymus (CT) DNA by a record value of 33.6 °C after 18 h incubation at a [PBD]:[DNA] ratio of 1:5.

Synthesis of the target molecule was initially approached using the thioacetal method of Thurston and co-workers.[9,10] However, this had to be abandoned due to the unwanted addition of EtSH across the C4-*exo*-methylene of intermediates of type **11** during attempted thioacetal formation. Instead, synthesis of **1** was achieved by employing the B-ring cyclisation





Scheme 1 *Reagents and conditions*: i, Alloc-Cl, aq. NaOH, THF, 0 °C, 87%; ii, MeOH, H_2SO_4, Δ, 43%; iii, $LiBH_4$, THF, 0 °C, 99%; iv, TBDMS-Cl, Et_3N, DBU, CH_2Cl_2, 52%; v, TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2, MeCN, 92% or $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −70 °C, 95%; vi, Ph_3PCH_3Br, KOBu^t, THF, 0 °C, 87%; vii, Bu_3SnH, $Pd(PPh_3)_2Cl_2$, H_2O, CH_2Cl_2, 77%; viii, $(COCl)_2$, DMF, THF, then **12**, Et_3N, H_2O, 0 °C, 74%; ix, TBAF, THF, 0 °C, 94%; x, $SnCl_2.2H_2O$, MeOH, Δ, 61%; xi, Alloc-Cl, pyridine, CH_2Cl_2, 0 °C, 50%; xii, TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2, MeCN, 32%; xiii, $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −45 °C, 51%; xiv, $Pd(PPh_3)_4$, PPh_3, pyrrolidine, CH_2Cl_2, MeCN, 0 °C, 77% for **1** and 43% for **19**.